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L5: Entry 6 of 6 File: USPT Apr 1, 1997

DOCUMENT-IDENTIFIER: US 5616500 A
TITLE: Trichohyalin and transglutaminase-3 and methods of using same

Brief Summary Paragraph Right (9):
In one aspect, the present invention comprises a purified molecule of DNA which having 20 or more consecutive nucleotides from SEQ ID NO:93, including a sequence that is homologous to SEQ ID NO:93 or complementary to SEQ ID NO:93, wherein SEQ ID NO:93 codes for the human trichohyalin gene. This DNA molecule can, in one embodiment, comprise the complete coding sequence of SEQ ID NO:93. Such a DNA molecule can also comprise a probe or primer selected from the group consisting of molecules having the sequences of SEQ ID NO:1 to SEQ ID NO:10. In yet another embodiment, the DNA molecule according to this aspect of the invention is present in a recombinant DNA vector, such as a plasmid. Such a vector can in turn be placed into a cell line which does not naturally contain the molecule of DNA. In another embodiment, the present invention comprises a molecule of RNA which can be translated in vitro or in vivo into the human trichohyalin protein. Such an RNA molecule comprises the coding sequence of SEQ ID NO:93, except that the thymine molecules of SEQ ID NO:93 are replaced by uracil molecules, including an RNA molecule having a sequence that is homologous or complementary to this sequence. Molecules of RNA which comprises 20 or more consecutive nucleic acids from such an RNA molecule are also included in the invention. In yet another embodiment, the invention comprises a purified protein molecule comprising 20 or more consecutive amino acids of the amino acid sequence of SEQ ID NO:94, including a protein molecule that is homologous to SEQ ID NO:94. In one embodiment, the protein molecule comprises the sequence of the human trichohyalin protein and contains the sequence of SEQ ID NO:94. Antibodies, such as monoclonal antibodies, having binding affinity for human trichohyalin and not for trichohyalin derived from other species are also included in this aspect of the invention.

Brief Summary Paragraph Right (11):
Another embodiment of this aspect of the invention includes a purified molecule of RNA which can be translated in vitro or in vivo into the human transglutaminase-3 protein and which comprises the coding sequence of SEQ ID NO:109, wherein the thymine molecules of SEQ ID NO:109 are replaced by uracil molecules. An RNA molecule having a sequence that is homologous or complementary to this sequence is also included. A purified molecule of RNA which comprises 20 or more consecutive nucleic acids from these RNA molecules is included as well. In another embodiment, the invention includes a purified protein molecule comprising 20 or more consecutive amino acids of the amino acid sequence of SEQ ID NO:112, including a protein molecule that is homologous to SEQ ID NO:112. Such a protein molecule can comprise the sequence of the human transglutaminase-3 protein, wherein the molecule comprises the sequence of SEQ ID NO:112. In a further embodiment, the invention also includes an antibody, such as a monoclonal antibody, having binding affinity for human transglutaminase-3 and not for transglutaminase-3 derived from other species.

Brief Summary Paragraph Right (13):
In a further embodiment, this aspect of the present invention includes a purified molecule of RNA which can be translated in vitro or in vivo into the mouse transglutaminase-3 protein and which comprises the coding sequence of SEQ ID NO:110, wherein the thymine molecules of SEQ ID NO:110 are replaced by uracil molecules, including an RNA molecule having a sequence that is homologous or complementary to this sequence. Also included is a purified molecule of RNA which comprises 20 or more consecutive nucleic acids from such an RNA molecule. In another embodiment, the

present invention comprises a purified protein molecule comprising 20 or more consecutive amino acids of the amino acid sequence of SEQ ID NO:111, including a protein molecule that is homologous to SEQ ID NO:111. Such a protein molecule can comprise the sequence of the mouse transglutaminase-3 protein, wherein the molecule comprises the sequence of SEQ ID NO:111. Also included in this aspect of the invention is an antibody, such as a monoclonal antibody, having binding affinity for mouse transglutaminase-3 and not for transglutaminase-3 derived from other species.

Drawing Description Paragraph Right (10):
FIG. 9A is a picture of pig TRHY which as been electrophoresed on a polyacrylamide gel. This picture reveals two bands of about 220 and 200 kDa that can be stained with coomassie blue (lane 1), and can also be detected by .sup.45 Ca binding (lane 2) or by use of a specific carboxyl-terminal epitope antibody (lane 3).

Detailed Description Paragraph Right (41):
(9) Residues 1850-1897: The carboxyl-terminal sequences are likely to adopt a folded or random coil conformation, due to the presence of prolines and glycines. Interestingly, the terminal 20 residues have been precisely conserved between sheep (5) and human, and have afforded the manufacture of a TRHY-specific antibody (Hamilton, E. H., et al., J. Invest. Dermatol. 98:881-889 (1992)).

Detailed Description Paragraph Right (44):
The sequence data of FIG. 3 and sequence homology data of FIG. 8 indicate the presence of two well-defined calcium binding domains of the EF-hand type. Prior to the present invention, methods were not described for the isolation of human epidermal or hair follicle TRHY. However, we show in FIG. 9A that pig tongue TRHY is capable of binding .sup.45 Ca in vitro (lane 2). Interestingly, unlike human, pig TRHY appears as two bands of about 220 and 200 kDa (lane 1), both of which bind calcium (lane 2). In addition, a Western blot using a new TRHY antibody (Hamilton, E. H., et al., J. Invest. Dermatol. 98:881-889 (1992)) elicited against the carboxyl-terminal 18 amino acids, which have been precisely conserved between human and sheep and presumably in pig TRHY as well, also reveals two bands of the same sizes (FIG. 9A, lane 3). Since these data indicate that the amino- and carboxyl-terminal ends have been conserved, this means that pig TRHY is expressed as two distinct protein products. By slot blotting (FIG. 9B), we show that pig TRHY (about 210 kDa, 2 EF-hands/mol) binds .sup.45 CaCl.sub.2 as effectively as calmodulin (14 kDa, 4 EF-hands/mol). Profilaggrin binds calcium somewhat more efficiently (Kozak, M. (1989), J. Cell Biol. 108, 229-241). Most of the calcium binding in the total epidermal extract is presumably due to the profilaggrin.

Detailed Description Paragraph Right (103):
At the simplest level, the amino acid sequence encoded by the foregoing polynucleotide sequences can be synthesized using commercially available peptide synthesizers. This is particularly useful in producing small peptides and fragments of larger polypeptides. (Fragments are useful, for example, in generating antibodies against the native polypeptide.)

Detailed Description Paragraph Right (109):
Since it may be necessary to purify and locate the transfected product, synthetic 15-mer peptides synthesized from the predicted cDNA sequence are injected into mice to generate antibody to the polypeptide encoded by the cDNA. The antibody can then be used to identify and purify the protein of interest by known methods.

Detailed Description Paragraph Right (110):
If antibody production is not possible, the cDNA sequence is additionally incorporated into eukaryotic expression vectors and expressed as a chimeric with, for example, .beta.-globin. Antibody to .beta.-globin is used to purify the chimeric. Corresponding protease cleavage sites engineered between the .beta.-globin gene and the cDNA are then used to separate the two polypeptide fragments from one another after translation. One useful expression vector for generating .beta.-globin chimerics is pSG5 (Stratagene). This vector encodes rabbit .beta.-globin. Intron II of the rabbit .beta.-globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression.

Detailed Description Paragraph Right (112):
Another aspect of the present invention comprises producing antibodies to the proteins of the present invention. Such antibodies can be used, for example, in assays for the detection of the proteins of the present invention.

Detailed Description Paragraph Right (113):
Substantially pure protein or polypeptide is isolated from the transfected or transformed cells as described above in Example 2. The concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the protein can then be prepared as follows:

Detailed Description Paragraph Right (114):
(1) Monoclonal Antibody Production by Hybridoma Fusion

Detailed Description Paragraph Right (115):
Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., Nature 256:495 (1975) or derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as Elisa, as originally described by Engvall, E., Meth. Enzymol. 70:419 (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. et al. Basic Methods in Molecular Biology Elsevier, N.Y. Section 21-2.

Detailed Description Paragraph Right (116):
(2) Polyclonal Antibody Production by Immunization

Detailed Description Paragraph Right (117):
Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than other and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. et al. J. Clin. Endocrinol. Metab. 33:988-991 (1971).

Detailed Description Paragraph Right (118):
Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. et al., Chap. 19 in: Handbook of Experimental Immunology D. Wier (ed) Blackwell (1973). Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12 .mu.M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: Manual of Clinical Immunology, 2d Ed. (Rose and Friedman, eds.) Amer. Soc. For Microbiol., Washington, D.C. (1980).

Detailed Description Paragraph Right (119):
Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample.

Detailed Description Paragraph Left (17):
B. Production of Antibodies

Detailed Description Paragraph Center (4):
Example 3: Producing Antibodies to the Proteins of the Present Invention

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L5: Entry 3 of 6 File: USPT Sep 28, 1999

DOCUMENT-IDENTIFIER: US 5958752 A
TITLE: Nucleic acid molecules encoding human trichohyalin and use thereof

Parent Case Paragraph Right (1):
This application is a divisional of U.S. patent application Ser. No. 08/056,200, filed Apr. 30, 1993, now U.S. Pat. No. 5,616,500.

Brief Summary Paragraph Right (8):
In one aspect, the present invention comprises a purified molecule of DNA which having 20 or more consecutive nucleotides from SEQ ID NO:93, including a sequence that is homologous to SEQ ID NO:93 or complementary to SEQ ID NO:93, wherein SEQ ID NO:93 codes for the human trichohyalin gene. This DNA molecule can, in one embodiment, comprise the complete coding sequence of SEQ ID NO:93. Such a DNA molecule can also comprise a probe or primer selected from the group consisting of molecules having the sequences of SEQ ID NO:1 to SEQ ID NO:10. In yet another embodiment, the DNA molecule according to this aspect of the invention is present in a recombinant DNA vector, such as a plasmid. Such a vector can in turn be placed into a cell line which does not naturally contain the molecule of DNA. In another embodiment, the present invention comprises a molecule of RNA which can be translated in vitro or in vivo into the human trichohyalin protein. Such an RNA molecule comprises the coding sequence of SEQ ID NO:93, except that the thymine molecules of SEQ ID NO:93 are replaced by uracil molecules, including an RNA molecule having a sequence that is homologous or complementary to this sequence. Molecules of RNA which comprises 20 or more consecutive nucleic acids from such an RNA molecule are also included in the invention. In yet another embodiment, the invention comprises a purified protein molecule comprising 20 or more consecutive amino acids of the amino acid sequence of SEQ ID NO:94, including a protein molecule that is homologous to SEQ ID NO:94. In one embodiment, the protein molecule comprises the sequence of the human trichohyalin protein and contains the sequence of SEQ ID NO:94. Antibodies, such as monoclonal antibodies, having binding affinity for human trichohyalin and not for trichohyalin derived from other species are also included in this aspect of the invention.

Brief Summary Paragraph Right (10):
Another embodiment of this aspect of the invention includes a purified molecule of RNA which can be translated in vitro or in vivo into the human transglutaminase-3 protein and which comprises the coding sequence of SEQ ID NO:109, wherein the thymine molecules of SEQ ID NO:109 are replaced by uracil molecules. An RNA molecule having a sequence that is homologous or complementary to this sequence is also included. A purified molecule of RNA which comprises 20 or more consecutive nucleic acids from these RNA molecules is included as well. In another embodiment, the invention includes a purified protein molecule comprising 20 or more consecutive amino acids of the amino acid sequence of SEQ ID NO:112, including a protein molecule that is homologous to SEQ ID NO:112. Such a protein molecule can comprise the sequence of the human transglutaminase-3 protein, wherein the molecule comprises the sequence of SEQ ID NO:112. In a further embodiment, the invention also includes an antibody, such as a monoclonal antibody, having binding affinity for human transglutaminase-3 and not for transglutaminase-3 derived from other species.

Brief Summary Paragraph Right (12):
In a further embodiment, this aspect of the present invention includes a purified molecule of RNA which can be translated in vitro or in vivo into the mouse transglutaminase-3 protein and which comprises the coding sequence of SEQ ID NO:110,

wherein the thymine molecules of SEQ ID NO:110 are replaced by uracil molecules, including an RNA molecule having a sequence that is homologous or complementary to this sequence. Also included is a purified molecule of RNA which comprises 20 or more consecutive nucleic acids from such an RNA molecule. In another embodiment, the present invention comprises a purified protein molecule comprising 20 or more consecutive amino acids of the amino acid sequence of SEQ ID NO:111, including a protein molecule that is homologous to SEQ ID NO:111. Such a protein molecule can comprise the sequence of the mouse transglutaminase-3 protein, wherein the molecule comprises the sequence of SEQ ID NO:111. Also included in this aspect of the invention is an antibody, such as a monoclonal antibody, having binding affinity for mouse transglutaminase-3 and not for transglutaminase-3 derived from other species.

Drawing Description Paragraph Right (10):
FIG. 9A is a picture of pig TRHY which as been electrophoresed on a polyacrylamide gel. This picture reveals two bands of about 220 and 200 kDa that can be stained with coomassie blue (lane 1), and can also be detected by .sup.45 Ca binding (lane 2) or by use of a specific carboxyl-terminal epitope antibody (lane 3).

Detailed Description Paragraph Right (29):
The sequence data of FIG. 3 and sequence homology data of FIG. 8 indicate the presence of two well-defined calcium binding domains of the EF-hand type. Prior to the present invention, methods were not described for the isolation of human epidermal or hair follicle TRHY. However, we show in FIG. 9A that pig tongue TRHY is capable of binding .sup.45 Ca in vitro (lane 2). Interestingly, unlike human, pig TRHY appears as two bands of about 220 and 200 kDa (lane 1), both of which bind calcium (lane 2). In addition, a Western blot using a new TRHY antibody (Hamilton, E. H., et al., J. Invest. Dermatol. 98:881-889 (1992)) elicited against the carboxyl-terminal 18 amino acids, which have been precisely conserved between human and sheep and presumably in pig TRHY as well, also reveals two bands of the same sizes (FIG. 9A, lane 3). Since these data indicate that the amino- and carboxyl-terminal ends have been conserved, this means that pig TRHY is expressed as two distinct protein products. By slot blotting (FIG. 9B), we show that pig TRHY (about 210 kDa, 2 EF-hands/mol) binds .sup.45 CaCl.sub.2 as effectively as calmodulin (14 kDa, 4 EF-hands/mol). Profilaggrin binds calcium somewhat more efficiently (Kozak, M. (1989), J. Cell Biol. 108, 229-241). Most of the calcium binding in the total epidermal extract is presumably due to the profilaggrin.

Detailed Description Paragraph Right (77):
At the simplest level, the amino acid sequence encoded by the foregoing polynucleotide sequences can be synthesized using commercially available peptide synthesizers. This is particularly useful in producing small peptides and fragments of larger polypeptides. (Fragments are useful, for example, in generating antibodies against the native polypeptide.)

Detailed Description Paragraph Right (83):
Since it may be necessary to purify and locate the transfected product, synthetic 15-mer peptides synthesized from the predicted cDNA sequence are injected into mice to generate antibody to the polypeptide encoded by the cDNA. The antibody can then be used to identify and purify the protein of interest by known methods.

Detailed Description Paragraph Right (84):
If antibody production is not possible, the CDNA Ad sequence is additionally incorporated into eukaryotic expression vectors and expressed as a chimeric with, for example, .beta.-globin. Antibody to .beta.-globin is used to purify the chimeric. Corresponding protease cleavage sites engineered between the .beta.-globin gene and the cDNA are then used to separate the two polypeptide fragments from one another after translation. One useful expression vector for generating .beta.-globin chimerics is pSG5 (Stratagene). This vector encodes rabbit .beta.-globin. Intron II of the rabbit .beta.-globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression.

Detailed Description Paragraph Right (86):
Another aspect of the present invention comprises producing antibodies to the proteins of the present invention. Such antibodies can be used, for example, in assays for the

detection of the proteins of the present invention.

Detailed Description Paragraph Right (87):
Substantially pure protein or polypeptide is isolated from the transfected or transformed cells as described above in Example 2. The concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the protein can then be prepared as follows:

Detailed Description Paragraph Right (88):
Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., Nature 256:495 (1975) or derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as Elisa, as originally described by Engvall, E., Meth. Enzymol. 70:419 (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. et al. Basic Methods in Molecular Biology Elsevier, New York. Section 21-2.

Detailed Description Paragraph Right (89):
Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected byamany factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than other and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. et al. J. Clin. Endocrinol. Metab. 33:988-991 (1971).

Detailed Description Paragraph Right (90):
Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. et al., Chap. 19 in: Handbook of Experimental Immunology D. Wier (ed) Blackwell (1973). Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12 .mu.M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: Manual of Clinical Immunology, 2d Ed. (Rose and Friedman, eds.) Amer.

Detailed Description Paragraph Right (92):
Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample.

Detailed Description Paragraph Center (34):
B. Production of Antibodies

Detailed Description Paragraph Center (36):
Producing Antibodies to the Proteins of the Present Invention

Detailed Description Paragraph Center (37):
(1) Monoclonal Antibody Production by Hybridoma Fusion

Detailed Description Paragraph Center (38):
(2) Polyclonal Antibody Production by Immunization

Detailed Description Paragraph Type 1 (29):
(9) Residues 1850-1897: The carboxyl-terminal sequences are likely to adopt a folded or random coil conformation, due to the presence of prolines and glycines. Interestingly, the terminal 20 residues have been precisely conserved between sheep (5) and human, and have afforded the manufacture of a TRHY-specific antibody (Hamilton, E. H., et al., J. Invest. Dermatol. 98:881-889 (1992)).

Related Application Patent Number (1):
5616500

Other Reference Publication (49):
Baden, H.P., Kubilus, J. & Phillips, S.B. (1987). "Characterization of Monoclonal Antibodies Generated to the Cornified Envelope of Human Cultured Keratinocytes". J. Invest. Dermatol. 89, 454-459.

L6 ANSWER 1 OF 5 MEDLINE
AN 97060439 MEDLINE
DN 97060439 PubMed ID: 8903474
TI Expression pattern of S100 calcium-binding proteins in human tumors.
AU Ilg E C; Schafer B W; Heizmann C W
CS Division of Clinical Chemistry, Department of Pediatrics, University of Zurich, Switzerland.
SO INTERNATIONAL JOURNAL OF CANCER, **(1996 Nov 4)** 68 (3) 325-32.
Journal code: GQU; 0042124. ISSN: 0020-7136.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199612
ED Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961223
AB The S100 Ca(2+)-binding proteins recently became of major interest because of their differential expression in neoplastic tissues, their involvement in metastatic processes, and the clustered organization of at least 10 S100 genes on human chromosome 1q21, a region frequently rearranged in several tumors. As a first attempt towards a specific and differentiated immunohistochemical classification of human tumors, we produced, purified and characterized a number of human recombinant S100 proteins and raised specific polyclonal **antibodies**. Their distinct cellular and intracellular localization was examined by immunohistochemical methods in normal and cancerogenic human tissues and cell lines. S100A1 and S100A2 can be detected in a few normal tissues only, whereas S100A4, S100A6, and **S100B** are expressed at higher levels in cancer tissues. In the future, these S100 **antibodies** will potentially be of great value in cancer diagnosis and therapy.

L6 ANSWER 2 OF 5 MEDLINE
AN 89012081 MEDLINE
DN 89012081 PubMed ID: 3172275
TI Major histocompatibility complex antigen expression on rat microglia following epidural kainic acid lesions.
AU Akiyama H; Itagaki S; McGeer P L
CS Department of Psychiatry, Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, Canada.
SO JOURNAL OF NEUROSCIENCE RESEARCH, **(1988)** 20 (2) 147-57.
Journal code: KAC; 7600111. ISSN: 0360-4012.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198811
ED Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19881116
AB Vigorous expression of major histocompatibility complex (MHC) class I and class I surface glycoproteins was observed on reactive microglia but not on astrocytes in the rat brain following lesions induced by epidural kainic acid (KA) on the cerebral cortex. The monoclonal **antibodies** used were OX18 against MHC class I, OX6 against MHC class II, OX1 against leukocyte common antigen (LCA), and W3/13 against pan-T lymphocytes. Astrocytes were marked by **antibodies** to glial fibrillary acidic protein (GFA) and **S100b** protein. The lesion differentially

affected four zones: the central area of the lesion where most cells died; the peripheral zone surrounding the lesion where selective damage occurred; projection tracts from the lesioned area; and terminal fields of damaged neurons. In nonlesioned animals, class I expression was confined to vascular endothelial cells and some small glial cells. Following KA treatment, class I-positive round cells appeared in the central zone at day 1, peaked about day 5, and then slowly declined. In the peripheral zone, class I-positive microglia were present fron day 2 on. They demonstrated classical morphology for such cells, and in some cases arranged themselves in pyramidal profiles surrounding neurons. Reactive microglia were also class I positive along tracts of damaged neurons and in the terminal areas. The reaction was reduced to control levels 16-20 weeks after lesioning although some vascular endothelial cells and a few round cells still stained positively in the cystic area, which was the remnant of the central zone. Class II antigen expression first appeared in the form of round cells in the central zone of the lesion on day 1. These peaked at 5-7 days and declined thereafter. In the peripheral zone on day 5, some positive round or ameboid cells were found intermingled with typical reactive microglia. This reaction peaked at about 1-2 weeks and decreased thereafter. Class II-positive microglia appeared in fiber tracts and in the terminal areas on day 5, peaked after 2-3 weeks, and declined thereafter. Double immunostaining for class I and II antigens showed that there were significantly fewer class II- than class I-positive cells, but the morphology of the two groups was similar. No astrocytes stained positively for either group I or group II antigen. In both the primary and secondary lesioned areas, LCA staining was observed on the surface of reactive microglia. In the primary lesions there were also LCA-positive round cells in the central zone, but these were rare in the peripheral zone and the secondary lesioned areas.(ABSTRACT TRUNCATED AT 400 WORDS)

L6 ANSWER 4 OF 5 MEDLINE
AN 86301451 MEDLINE
DN 86301451 PubMed ID: 3743875
TI Evidence that S100 proteins regulate microtubule assembly and stability in
rat brain extracts.
AU Hesketh J; Baudier J
SO INTERNATIONAL JOURNAL OF BIOCHEMISTRY, **(1986)** 18 (8) 691-5.
Journal code: E4S; 0250365. ISSN: 0020-711X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198610
ED Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19861022
AB Microtubule re-assembly in rat brain extracts was inhibited by **antibodies** to S100 proteins. Anti-S100 **antibodies** caused an increase in the cold-stability of microtubules and this effect was abolished by the presence of short lengths of microtubules formed under control conditions. Anti-S100 **antibodies** had no effect on the stimulation of assembly or the increase in microtubule stability caused by low zinc concentrations. Addition of exogenous S100a and **S100b** to brain extracts had different effects on assembly; S100a caused an inhibition of assembly while **S100b** stimulated the early phase of assembly. The data suggest that endogenous **S100b** is involved in the regulation of microtubule assembly in brain extracts.

L10 ANSWER 2 OF 53 MEDLINE
AN 96182331 MEDLINE
DN 96182331 PubMed ID: 8632055
TI Immunoreactive **S100** proteins of blood immunocytes and **brain** cells.
AU Singh V K; Cheng J F
CS Department of Psychiatry, University of Michigan, Ann Arbor 48109 USA.
SO JOURNAL OF NEUROIMMUNOLOGY, **(1996 Feb)** 64 (2) 135-9.
Journal code: HSO; 8109498. ISSN: 0165-5728.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199607
ED Entered STN: 19960715
Last Updated on STN: 19970203
Entered Medline: 19960702
AB **Brain S100**, an acidic protein with Ca2+-binding and neurotrophic properties, may be involved in the genesis of neurodegenerative diseases. Based on sharing of common antigens between the immune and nervous systems, we performed a comparative analysis of **S100** in blood immunocytes (lymphocytes and monocytes) and **brain** cells. By using polyclonal **antibodies** to **S100**, an immunoreactive **S100** was detected in human blood immunocytes and U373 human astrocytoma cells. The U373 cells contained a much higher level of **S100** as compared to immunocytes, both cell types being compared at 1 X 10(6) cell concentration. Through protein-immunoblotting, the immunocyte antigen was compared with pure **S100** of bovine **brain** (authentic sample) and **S100** of U373 cells and **brain** cells (human and mouse **brain**). The monomeric form of immunocyte-derived **S100** was a low molecular mass (12-14kDa) protein, but slightly larger than authentic **S100** (10.5 kDa) The **S100** of U373 cells and **brain** cells was mainly a polymer (60-100 kDa), although the **brain** cells also showed a low molecular mass (10.5 kDa) band that corresponded to authentic **S100**. The molecular mass differences suggest that peripheral blood immunocytes contain an immunoreactive **S100** that differs in size but is antigenically related to **brain** **S100** family.

L12 ANSWER 4 OF 4 MEDLINE
AN 85014901 MEDLINE
DN 85014901 PubMed ID: 6207537
TI Production and characterization of monoclonal **antibodies** with specificity for the **S100** beta polypeptide of **brain** **S100** fractions.
AU Van Eldik L J; Ehrenfried B; Jensen R A
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1984 Oct)** 81 (19) 6034-8.
Journal code: PV3; 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198411
ED Entered STN: 19900320
Last Updated on STN: 19970203
Entered Medline: 19841119
AB **S100** refers to a heterogeneous fraction of low-molecular-weight acidic calcium-binding proteins. We report here production and characterization of two mouse hybridomas that secrete monoclonal **antibodies** that appear to be specific for the **S100** beta polypeptide of **brain** **S100** preparations. By **ELISA**, RIA, and immunoblotting analysis, the monoclonal **antibodies** react specifically with **S100** beta and show little or no reactivity with any **S100** alpha-like polypeptides. In addition, there is no reactivity with the structurally homologous proteins calmodulin and troponin C. The utility of these monoclonal **antibodies** for immunocytochemical studies of clinical pathology specimens has been demonstrated by examination of **S100** beta localization in human autopsy **brain** and anaplastic astrocytoma sections. **S100** beta is localized primarily in glial cell cytoplasm and processes, with no specific staining observed in glial cell nuclei, erythrocytes, or neuronal cells. These monoclonal **antibodies** may have important applications in pathological examination of surgical specimens as a specific marker for tumors containing **S100** beta, will allow a more precise interpretation of the distribution and localization of **S100** beta in both normal and neoplastic tissues, and may provide insight into the physiological functions of the **S100** proteins.

L9 ANSWER 9 OF 12 MEDLINE
AN 96188414 MEDLINE
DN 96188414 PubMed ID: 8608198
TI Measurement of **S-100** protein in human blood and cerebrospinal fluid: analytical method and preliminary clinical results.
AU Missler U; Wiesmann M
CS Neuroradiologie am Institut fur Radiologie der Medizinischen Universitat zu Lubeck, Germany.
SO EUROPEAN JOURNAL OF CLINICAL CHEMISTRY AND CLINICAL BIOCHEMISTRY, (1995 Oct) 33 (10) 743-8.
Journal code: 9105775. ISSN: 0939-4974.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199605
ED Entered STN: 19960605
Last Updated on STN: 19960605
Entered Medline: 19960528
AB An immunofluorometric **sandwich** assay for determination of **S-100** protein in cerebrospinal fluid (CSF) and blood is described. The lower detection limit was 0.015 micrograms/l of **S-100** protein. Intra-assay and inter-assay imprecision (coefficients of variation, CVs) were 2.1 to 3.2% and 7.8 to 11.6%, respectively. **S-100** protein recovery in cerebrospinal fluid was 94 to 103%. In blood the recovery varied from 67 to 96%, depending on blood samples used and the concentration of **S-100** protein. The best recovery in blood was found using heparinized plasma. In healthy subjects 0.098 +/- 0.11 micrograms/l (mean +/- SD) of **S-100** protein was detected (n = 30). In the CSF of otherwise healthy patients undergoing a myelography for lumbar pain 1.43 +/- 0.49 micrograms/l (mean +/- SD) of **S-100** protein was found. Preliminary results from longitudinal studies on **S-100** protein in neurosurgical patients indicate a positive correlation between **S-100** protein blood levels and clinical course. Thus, the determination of **S-100** protein in blood appears to be helpful in the monitoring of patients with neuronal damage.

L9 ANSWER 8 OF 12 MEDLINE
AN 97380114 MEDLINE
DN 97380114 PubMed ID: 9236913
TI A specific and sensitive ELISA for measuring S-100b in cerebrospinal fluid.
AU Green A J; Keir G; Thompson E J
CS Department of Neuroimmunology, National Hospital for Neurology and Neurosurgery, London, UK.. skgtejt@ion.bpmf.ac.uk
SO JOURNAL OF IMMUNOLOGICAL METHODS, (1997 Jun 23) 205 (1) 35-41.
Journal code: 1305440. ISSN: 0022-1759.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199708
ED Entered STN: 19970908
Last Updated on STN: 19970908
Entered Medline: 19970822
AB A sensitive, simple and specific **sandwich** ELISA for S-100b is described. This method involves the binding of a monoclonal anti-S-100b antibody to the wall of a microtitre plate. This capture antibody is subsequently incubated with S-100b standard, control or patient sample in the form of cerebrospinal fluid (CSF). After incubation, the microtitre plate is washed and horseradish peroxidase-labelled polyclonal anti-S-100b is added (detector antibody). The amount of detector antibody bound to the microtitre plate is proportional to the amount of S-100b in the sample. The assay has a lower limit of detection of 0.04 ng/ml and shows < 0.006% reactivity with the closely related polypeptide S-100a. The assay has a mean within-batch precision of 9.3 and 5.6% at S-100b concentrations of 0.38 and 0.8 ng/ml, respectively. The between batch precision is 8.9 and 8.1% at S-100b concentrations of 0.12 and 0.34 ng/ml, respectively. The recovery of S-100b from CSF spiked with 0.5 ng/ml was 94% with a CV of 8.5%. The assay may be completed in less than 5 h using precoated microtitre plates, thus lending itself to routine use in clinical laboratories. Using this ELISA, 154 CSF samples were analysed and 19% of samples were found to have elevated levels. The highest levels were found in patients with cerebral haemorrhage or central nervous system malignancy. S-100b concentrations from individuals without evidence of neurological disease were found to be less than 0.4 ng/ml. Only 5% of patients with multiple sclerosis were found to have elevated CSF S-100b concentrations. Serial CSF samples taken from a patient with an infected in-dwelling shunt showed a dramatic decline, suggesting that S-100b is rapidly cleared.

L9 ANSWER 7 OF 12 MEDLINE
AN 97380601 MEDLINE
DN 97380601 PubMed ID: 9237376
TI A **sandwich** enzyme immunoassay for brain **S-100** protein and its forensic application.
AU Seo Y; Kakizaki E; Takahama K
CS Department of Legal Medicine, Miyazaki Medical College, Japan.
SO FORENSIC SCIENCE INTERNATIONAL, (1997 Jun 6) 87 (2) 145-54.
Journal code: 7902034. ISSN: 0379-0738.
CY Ireland
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199709
ED Entered STN: 19970916
Last Updated on STN: 19970916
Entered Medline: 19970903
AB A sensitive **sandwich** enzyme immunoassay for identification of brain **S-100** protein in blood or bloodstains containing brain tissue is described. A polystyrene ball coated with rabbit anti-**S-100** protein IgG was incubated with human **S-100** protein, and then with anti-**S-100** Fab'-peroxidase conjugate. Peroxidase activity bound to the polystyrene ball was assayed by fluorometry using 3-(4-hydroxyphenyl)propionic acid as the hydrogen donor. The detection limit of human **S-100** protein was 0.6 pg (30 amol) per assay tube. The cross-reaction of this **sandwich** enzyme immunoassay to other organs was approximately 1/100 or less. Antigenic activity of **S-100** protein in bloodstains containing brain extracts was detectable after storage for 36 days at room temperature. The ratio of **S-100** protein to total protein (ng/mg) in bloodstains when brain tissue was mixed with normal human blood at concentrations of 5-500 mg/ml was approximately 100-fold those of other samples (liver, heart, intestine, and skeletal muscle). These results indicated that bloodstains mixed with brain tissue were clearly distinguishable from others. Thus, in forensic practice, measurement of **S-100** protein or the ratio of **S-100** protein to total protein is useful to identify blood and bloodstains containing brain tissue.

L7 ANSWER 40 OF 41 MEDLINE
AN 85109453 MEDLINE
DN 85109453 PubMed ID: 2578587
TI Production of **monoclonal antibodies** directed against antigenic determinants common to the alpha- and beta-chain of bovine brain S-100 protein.
AU Vanstapel M J; Peeters B; Cordell J; Heyns W; De Wolf-Peeters C; Desmet V; Mason D
SO LABORATORY INVESTIGATION, **(1985 Feb)** 52 (2) 232-8.
Journal code: 0376617. ISSN: 0023-6837.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198503
ED Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19850313
AB BALB/c mice were immunized with S-100 protein, which was isolated from bovine brain. The first fusion resulted in the cloning of three stable hybridoma lines (S1-61-64, S1-61-65, S1-87-4) that produced **monoclonal antibodies** against S-100 protein. The hybridoma lines obtained from a second fusion (S2-20, S2-95) were not stable and **antibody** production ceased early during cloning. Immunoblotting results showed that all **antibodies** reacted with antigenic determinants shared by both the alpha- and **beta** -subunit of **S-100** protein. These antigenic sites appeared to differ from the calcium-binding site since immunoblotting against other calcium-binding proteins sharing this site (calmodulin, carp parvalbumin, oncomodulin) was negative. Despite the fact that the immunoblotting reactions of the **antibodies** obtained from both fusions were indistinguishable, different immunohistologic labeling patterns could be observed. These **antibodies** have proven to be excellent reagents for the immunocytochemical detection of S-100 in normal and pathologic human tissue.

L7 ANSWER 37 OF 41 MEDLINE
AN 86279545 MEDLINE
DN 86279545 PubMed ID: 3734419
TI Immunohistochemical localization of **S100 beta** in human nervous system tumors by using **monoclonal antibodies** with specificity for the **S100 beta** polypeptide.
AU Van Eldik L J; Jensen R A; Ehrenfried B A; Whetsell W O Jr
SO JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY, **(1986 Aug)** 34 (8) 977-82.
Journal code: 9815334. ISSN: 0022-1554.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198609
ED Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19860917
AB The immunohistochemical localization of the calcium-binding protein, **S100 beta**, in human nervous system tumors has been examined by using a **monoclonal antibody** with specificity for the **S100 beta** polypeptide. **S100 beta**-specific immunoreactivity is detected in astrocytoma, glioblastoma, Schwannoma, ependymoma, and craniopharyngioma, whereas no reactivity is seen in oligodendroglioma, meningioma, neuroblastoma, or medulloblastoma. These data suggest that analysis of **S100 beta** localization with these **monoclonal antibodies** may be useful for research or diagnostic purposes.

L7 ANSWER 1 OF 41 MEDLINE
AN 96383407 MEDLINE
DN 96383407 PubMed ID: 8791271
TI Prognostic value of serum analyses of **S-100 beta** protein in malignant melanoma.
AU von Schoultz E; Hansson L O; Djureen E; Hansson J; Karnell R; Nilsson B; Stigbrand T; Ringborg U
CS Department of Oncology, Radiumhemmet, Karolinska Hospital, Stockholm, Sweden.
SO MELANOMA RESEARCH, **(1996 Apr)** 6 (2) 133-7.
Journal code: 9109623. ISSN: 0960-8931.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199611
ED Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961107
AB S-100 protein was first described in the central nervous system but is also present in malignant melanoma cells. Immunohistochemical detection of S-100 is widely used in the histopathological diagnosis of malignant melanoma. In the present study serum levels of **S-100 beta** protein were measured in 643 patients with cutaneous malignant melanoma. An immunoradiometric assay with three **monoclonal antibodies** against bovine **S-100** protein **beta** subunit was used. At the time of blood sampling 553 patients were in clinical stage I, 24 in clinical stage II and 66 in clinical stage III. The overall survival rate was strongly associated with serum levels of S-100 protein. The observed/ expected death ratio was markedly increased with increasing levels of **S-100 beta** ($P < 0.001$). A fivefold increase in relative hazard was indicated by a value of **S-100 beta** exceeding 0.6 microgram/l ($P < 0.001$) and when this cut-off level was used **S-100 beta** had additional prognostic value independent of clinical stage ($P < 0.001$). Our data strongly suggest that **S-100 beta** in serum is an independent prognostic marker that may be useful in identifying high-risk cases and monitoring response to therapy in patients with malignant melanoma.

L7 ANSWER 2 OF 41 MEDLINE
AN 96126687 MEDLINE
DN 96126687 PubMed ID: 8574684
TI **S-100 beta** has a neuronal localisation in the rat hindbrain revealed by an antigen retrieval method.
AU Yang Q; Hamberger A; Hyden H; Wang S; Stigbrand T; Haglid K G
CS Department of Anatomy and Cell Biology, University of Goteborg, Sweden.
SO BRAIN RESEARCH, **(1995 Oct 23)** 696 (1-2) 49-61.
Journal code: 0045503. ISSN: 0006-8993.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199603
ED Entered STN: 19960321
Last Updated on STN: 19960321
Entered Medline: 19960314
AB The localisation of S-100 in mammalian CNS neurons has been under debate for more than two decades. We address the question with two polyclonal and two new **monoclonal antibodies**. The specificity and the distribution in rat brain is based on an antigen retrieval method. We present evidence that aldehyde fixatives mask **S-100 beta** in neurons, and that the immunoreactivity is retrieved after trypsinisation. Neuronal **S-100 beta** is also detected in unfixed and ethanol fixed sections. The neuronal immunoreactivity is partly solubilised from unfixed tissue sections with 2.5 mM EDTA and is completely extracted with 2.5 mM EDTA and 1% Triton X-100. Most of the glial **S-100 beta** is washed out from unfixed tissue sections with saline. **S-100 beta** has distinct distribution in neurons of the hindbrain, i.e., the brainstem and cerebellum, but is not observed in the forebrain. One of the **monoclonal antibodies** immunostained neither neurons nor glia when it had been absorbed with S-100 crosslinked to nitrocellulose membranes. The distribution of neuronal **S-100 beta** differed from that of other neuronal calcium binding proteins, such as calbindin and parvalbumin. It was confined mainly to cholinergic neurons of the hindbrain. The presence of **S-100 beta** in distinct neuronal populations may indicate neurotrophic effects of **S-100 beta**. The notion is supported by the capability of S-100 to cause neurite outgrowth in vitro.